REMARKS

I. Status of the Claims

Claims 1-14, 16-20, 26-32, 36, and 37 are pending. There are no pending rejections given that the Board has vacated all of the rejections prior to remanding the application. Claims 1-6, 16, 18-19, 26, 28, 36 and 37 have been amended. Claims 146-150 have been added. A copy of the claims as amended is shown in Appendix A. An unofficial clean copy of the pending claims is shown in Appendix B for convenience. Applicants provide the following remarks for consideration by the examiner.

II. Claim Amendments

Claim 1 was amended to read "inhibiting growth of a tumor cell expressing wild-type p53" to clarify that "p53-positive" tumor cells express the wild-type p53 protein. Wild-type p53 is further defined in the Specification section, page 8, lines 4-5. Claim 1 was also narrowed to specify "human" subjects rather than just "mammalian" subjects by introducing the subject matter of canceled claim 6 into claim 1. Furthermore, "directly administering" was amended to read "parenterally administering" in order to clarify the precise meaning of "direct." "Parenteral" is defined as "taken into the body or administered in a manner other than through the digestive tract, as by intravenous or intramuscular injection" (ref. www.dictionary.com, attached). Support for parenteral administration can be found in greater detail in the Specification section, page 32, lines 8-10. The Applicant broadened the administration of the p53-adenoviral construct to incorporate injection into the "subject" rather than just minimizing the injection site into the "tumor *in vivo*". This is further explained on page 32, lines 6-8 wherein it states that "Administration of therapeutic compositions according to the present invention will

be via any common route so long as the target tissue is available via that route". For example, this could occur via "intravenous or intraarterial routes" (page 33, line 19) that leads to the tumor site rendering the therapeutic application effective. The Applicant also found that "wherein said tumor comprises cells that express a functional p53 polypeptide" is redundant because the above amendments to claim 1 incorporate the fact that the tumor cells comprise wild-type p53 which gives rise to these cells being functional. Additionally, assays have been previously established within the scientific community (*i.e.*, immunohistochemistry and/or DNA sequencing) that tests for the presence of wild-type p53 in tumor cells.

New claim 146 has been introduced to incorporate a "method of inducing apoptosis in a tumor cell expressing wild-type p53". This evidence is supported by Example 3 (pages 56-60). Previous research has shown that wild-type p53 plays an important role in apoptosis (page 2, lines 26-28). Therefore, administration of a viral vector expression system containing wild-type p53 would not only have a pronounced effect on the growth of a tumor cell but also on that tumor cell's ability to undergo apoptosis.

Dependent claims 2-6, 16, 19, 26, 28 and 36 have been placed into multiple dependent claim format. They were changed to incorporate not only "a method of inhibiting growth of a tumor cell" (claim 1) but also "a method of inducing apoptosis" (claim 146) post-administration of a "viral expression construct" containing a functional p53 polypeptide.

Claim 18 was amended to focus on the definition of a "body cavity" since it was not previously defined. Further explanation of an "artificial body cavity" can be found on page 35 in the Specification section.

Claims 26 and 38 were changed from "contacting" to "administering" based on "administering" being the antecedent in the Specification (page 34, line 13).

II. The Board Erred in Finding that Katayose and Srivastave were Relevant to Appealed Rejections

Applicants previously submitted a declaration under Rule 131 to antedate publications by Katayose *et al.* ("Katayose") and Srivastava *et al.* ("Srivastava"). That declaration was based on the possession, by the inventors, of the same data presented in the Katayose and Srivastava papers prior to publication of the data by those authors. In response, the examiner withdrew the rejection based on those references.

On appeal, the Board argued that this showing was factually insufficient to establish that Katayose and Srivastava were not available as prior art against the present invention. Based on this holding, the Board found that "Katayose and Srivastava both show positive results using the adenoviral vector" and "[t]hus, Katayose and Srivastava appear to be very relevant to the issue of patentability of the instant claims under 35 U.S.C. §103." Applicants could not disagree more.[1]

A. Katayose and Srivastava Teach Away

As explained in great detail in the prosecution, Katayose and Srivastava fail to render obvious the present invention. The basis for these conclusions was explained numerous times, including in Applicants Appeal and Reply Briefs. To make this submission complete, Applicants again provide the substance of those arguments for review:

> A more extreme example was provided in appellant's brief, where it was noted that Srivastava disclosed "[a] recent study [which] described an intriguing result in which an adenovirus-p53 expression vector did not inhibit the *in vitro* growth of a metastatic variant of LNCaP cells; however, the growth of these cells was inhibited *in vivo*.[31]" This is additional evidence that there is ***considerable*** unpredictability as to whether *in vitro* results will be the same as those in an *in vivo* environment.

[1] It is noted that Srivastava was published in December, 1995, and thus is not even available as prior art to the present case under §102(a). Even if it were available as prior art, Srivastava would still not be relevant for the various reasons that have been previously set forth and enumerated herein.

However, the examiner attempts to circumvent this argument on the basis that the p53 field is different, namely, in that there was a demonstrated correlation between *in vitro* studies and *in vivo* animal models at the time the present application was filed. However, any such correlation related to p53-***deficient*** cells. Thus, it would be necessary, in relying on this showing, to have some basis for equating the treatment of p53-positive and p53-negative cells. It is clear that the conventional thinking, as of Appellant's priority, was that p53 ***replacement*** was capable of restoring growth regulation to cells lacking that function. It was far less clear that p53 ***supplementation*** would have any real benefit.

As argued previously, there was considerable confusion in the field regarding gene therapy p53-positive tumors. For example Katayose stated that "tumor cells that were null for p53 prior to infection ... and tumor cells that expressed mutant endogenous p53 protein ... were more sensitive to AdWTp53 cytotoxicity than cells that contained the wild-type p53 ..," and that "these studies indicated that an adenovirus vector expressing wild-type p53 is markedly cytotoxic ***to tumor cells that have null or mutant p53 expression*** ..." (emphasis added). In addition, the last line of the abstract summarizes the authors conclusions: "These data suggest that endogenous p53 status is a determinant of AdWTp53-mediated cell killing of human tumors." The clear inference is that only p53 null or p53 mutant tumor cells are killed by AdWTp53, not tumor cells that are WTp53.

Katayose indeed actually teaches away from treating p53-positve tumor cells with p53 expression vector. In the Discussion on page 896, first column, second paragraph, it is stated that "There are several possible mechanisms by which high expression of wild-type p53 results in apoptosis in tumor cells devoid of p53 or expressing mutant p53, but not in tumor or normal cells expressing wild-type p53". Thus, Katayose is itself stating quite clearly that expression of wild-type p53 would not be expected to effect apoptosis in a tumor which expresses wild-type p53.

The following additional comments also illuminate what the skilled artisan would take away from Katayose:

> "As shown in Fig. 3, *A* and *B*, infection of H-358 and MDA-MB-231 [p53 null and mutant, respectively] cells with AdWTp53 completely inhibited cell growth .... In contrast, MCF-7 cells [p53 positive] continued to proliferate although at a slower rate than control cells ...."

Page 892, right hand column.

> "It appears that cells that express wild-type p53 were 5-250 times more resistant to the AdWTp53-mediated inhibitory effect on cell growth when compared with cells expressing no *p53* or mutant *p53*."

Page 893, right hand column.

> "These results indicate that tumor cells null for *p53* or expressing an endogenous mutant *p53* undergo apoptosis following exposure to AdWTp53, whereas tumor cells or normal cells expressing wild-type p53 are resistant to apoptosis."

Page 895, right hand column.

> "... [O]verexpression of wild-type p53 induced programmed cells death (apoptosis) of tumor cells devoid of wild-type p53 or expressing endogenous mutant *p53*, but not in tumor or normal cells expressing wild-type p53."

Page 896, left hand column.

These passages clearly indicate that the Katayose reference cannot be read as providing sufficient motivation for treating p53-positive cells. To the contrary, the reference suggests the opposite, that p53-positive cells are far less susceptible to such treatments. Unfortunately, the examiner failed to provide any response to these arguments.

Srivastava also provides an insufficient basis for suggesting that one should ***clinically*** treat p53-positive cells with a p53 expression construct. In fact, they state that "in agreement with the previous observations,[18] we also did not detect a growth inhibitory effect of AdWTp53 on breast cancer cells, MCF7 containing endogenous wt p53 (data not shown)." No clearer statement to counter the examiner's position could be imagined.

The examiner's response to this argument glosses over the MCF7 issue and turns instead to DU145- and PC3-derived cells as showing predictability for *in vivo* applications. This misses the point entirely. MCF7 has wild-type p53; the other cells lines do not. The reference, therefore, suggests to the skilled artisan that p53 gene therapy of p53-positive cells will ***not*** be successful. The Answer fails entirely to address this issue.

Reply Brief at pages 10-14. Thus, on the record presented to it, the Board could have and should have decided that the present claims were ***not*** obvious, with or without reference to Katayose and Srivastava.

B. The Examiner Has Not Shown that In Vitro Studies Involving WT p53 Tumor Cells Are Relevant to Human Therapy

Further, to support any rejection on the basis of the studies shown in Katayose or Srivastava, the Examiner is required to demonstrate on the record that one of skill in the art would have believed that these *in vitro* studies of wild-type tumors were somehow reasonably

predictive of success in human cancer patients. The Examiner has made no such showing. Indeed, it is well-known within the scientific community that animal studies are necessary for the advancement of science both in understanding human physiology and in identifying possible treatments to human diseases. However, while animal models are convenient and necessary, they are not always an accurate representation as to what occurs in humans. One example is that human cystic fibrosis pathology results from a mutation in the CFTR gene causing severe respiratory ailments (*Pathology*, Ed. 2, E. Rubin and J. L. Farber, J. B Lippincott Co., Philidelphia, 1994, p.231-234, copy enclosed). A mouse model of this same mutated CFTR gene causes specific problems within the digestive tract and no significant problems to the mouse respiratory system (Ameen *et al.*, *Histochem Cell Biol*, 114(1), 2000, copy enclosed). Thus, the same genes in animal models and humans do not necessarily, and in fact often do not, play the same role in disease progression and treatment.

Furthermore, the Patent and Trademark Office's position has consistently been that *in vitro* studies and animal models of various human diseases are not a reliable indicator of the therapeutic benefit of treatments in humans as cited in Crystal (*Science*, 270, 1995, 404-410, copy enclosed), Gomez-Navarro *et al.* (*European J. of Cancer*, 35(6), 1999, 867-885, copy enclosed) and Sigmund (*Arterioscler. Thromb. Vasc. Biol.*, 20, 2000, 1425-1429, copy enclosed). Crystal notes that "[t]here have been several surprise examples, in which predictions from gene transfer studies in experimental animals have not been borne out in human safety and efficacy trials" (p. 409). This sentiment is echoed in Gomez-Navarro *et al.* which states that the successful treatments found in animal models are not consistently found in humans (p. 875). Additionally, Sigmund expresses concern for the various findings within the same species, only on different genetic backgrounds (p. 1425).

The references cited by the Examiner do not refer to any human clinical findings, only to findings within animal model systems (specifically the nude mouse model). However, Applicant has provided scientific proof of human clinical data on the benefits of the wild-type p53 viral vector expression system in suppressing growth of HNSCC, thereby rendering the Examiner's arguments as null and void. The mere fact that animal and *in vitro* studies have been found reasonably predictive in the case of therapy of tumors bearing mutant p53 genes, there is no indication on ***this*** record that such studies would be reasonably predictive of success in treating animals having wild-type p53 tumors.

C. The Claims are Now Limited to Human Therapy

Furthermore, Katayose and Srivastava are particularly irrelevant to the subject matter of new claim 1 (which introduces the subject matter of previous claim 6) for several reasons cited previously in great detail in Section II. These reasons include contradictory data between their *in vitro* and *in vivo* studies as well as teaching away from using Ad-WTp53 in tumor cells that contain wild-type p53 claiming that there is no effect on these cell types. Most importantly, these references are inappropriate because of their specific reference to *in vitro* experiments and not human treatment. Both Katayose and Srivastava perform all of their experiments *in vitro* on various human cancer cell lines. The results derived from these *in vitro* experiments are less dependable than animal model studies for the effective therapeutic benefit of human treatment and as a result are not an accurate representation of the effects of human treatment. Therefore, it is the burden of the Examiner to provide substantial evidence that is reasonably predictive to human clinical therapy of wild-type p53 tumors. There has been no showing by the Examiner as to the predictive quality of these references.

D. The Rule 131 Declaration Shows that Dr. Clayman Had Obtained an IND and Initiated the Human Clinical Trial Process Before the Publication of Katayose or Srivastava

Applicants have already shown that the references of Katayose and Srivastava are not relevant so there is no need to submit a new declaration under 37 C.F.R. §1.131. Nevertheless, Applicants are providing a declaration under Rule 131 directed towards documentation that the inventor was involved in setting up and performing clinical trials in human patients directed at the study of the effects of Ad-WTp53 gene therapy on both wild-type and mutated p53 tumor cells during critical time periods. Applicants hereby submit a new declaration from the inventor under Rule 131. This declaration avers conception of the present invention prior to August 11, 1995, the date of the earliest publication as between Katayose and Srivastava, and diligence to Applicant's filing date in November, 1995. Within the declaration, the following documents dated prior to August 11, 1995 were cited in support of conception:[2]

1. A transcribed tape of a Grand Rounds Seminar citing the success in treating both wild-type and mutated p53 in tumor cells in animals (paragraph 5A of the Clayman Declaration, Exhibit 1) and indicating that his focus is on the development of novel molecular therapies for treating cancer. Please note that **bolded** portions of this presentation which indicate his findings that the p53 therapy worked to slow or inhibit tumor growth in both cellular and in animal models of human tumors. It should be noted that the date of this Grand Rounds presentation to a group of clinicians at MD Anderson was more than one year prior to the November, 1995 filing date. It included the showing of slides, but no handouts were given out and there was no abstract of this presentation prepared or published.

[2] The attachments have all been redacted to remove certain names and also to remove all dates prior to August 11, 1995. All of the date redactions have been designated with an "*".

2. There is a document dated prior to August 11, 1995 demonstrating approval from the Food and Drug Administration ("FDA") and the Institutional Review Board of MD Anderson Cancer Center ("IRB") for the use of adenovirus treatment and for the initial protocol submission (paragraph 5C, Exhibit 2).

3. The final version of the approved informed consent form (paragraph 5D, Exhibit 3)

4. The final version of the protocol used in the clinical study indicating Ad-WTp53 induced apoptosis in tumor cells regardless of p53 status is attached as Exhibit 4 to the Clayman declaration, and discussed in paragraphs 5E and 5F of the declaration. In this protocol, Dr. Clayman observes that his laboratory studies had shown that head and neck squamous cell carcinomas ("HNSCC") underwent apoptosis (cell death) when treated with Ad-WTp53, regardless of endogenous p53 status, and indicates that "[t]hese results support the use of this strategy in a clinical trial." See Protocol, page 3, second full paragraph. For this reason, the study was designed to include patients having HNSCC regardless of p53 endogenous status of the tumor, and to assess the tumor for its p53 status. See, *e.g.*, Protocol, page 11, section 6.7.

5. Additional documents were cited to prove diligence after conception: Approval from the FDA for the Investigational New Drug ("IND") of Ad-WTp53 (5G, Exhibit 5); various documents inquiring and granting approval for revised versions of the protocol used in the clinical study (paragraphs 5H-J, Exhibits 5-8); documentation showing IRB approval for the clinical study (5K, Exhibit 9); and treatment of patients (5L). Also, this declaration highlights the irrelevancy of the Katayose and Srivastava references due to their lack of experiments involving human subjects, their inconsistencies within their own data sets, and the fact that they

teach away from examining wild-type p53 tumors. In light of this declaration, Applicants again submit that Katayose and Srivastava are not properly citable against the present invention.

III. Comments on Previous Rejections and Applied to Amended Claims

The examiner's previous obviousness rejection, vacated by the Board, was of claims 1-14, 16-20, 26-32, 36 and 37 over Cajot taken with Wills or Liu, in view of Zhang or Bramwell. Applicants have argued throughout the prosecution that extrapolation from *in vitro* to *in vivo* studies is problematic at best. With respect to Cajot *et al.* in particular, it was argued that these studies are unrelated to the actual therapy of human tumors, and are technically flawed. The Baker *et al.* (Exhibit J), Casey *et al.* (Exhibit K), Katayose *et al.* (Exhibit F) and Srivastava *et al.* (Exhibit G) references have been argued to evidence confusion in the art. And finally, Applicant has provided experimental data showing surprising and unexpected results flowing from practice of the present invention.

In return, the examiner has argued that "there has been no rejection on the record that extrapolating from *in vitro* to *in vivo* inhibition of tumor cells expressing p53 is problematic or unpredictable." Issues of extrapolation are said to be fact specific, and in the instant case, Wills and Liu establish that extrapolating from *in vitro* to *in vivo* is appropriate.

The examiner also argues that Baker *et al.* (Exhibit J) and Casey *et al.* (Exhibit K) do not, as alleged by Applicants, evidence confusion in the field. To the contrary, the examiner argues that these references support the notion that overexpression of p53 can inhibit growth of a wide variety of cells, including p53-positive tumor cells.

Next, the examiner disputed that Cajot is flawed, arguing that because SV40 "seems to be the least affected" by p53, the skilled artisan would be motivated to use this promoter in studies

and, further, that the limitations on Cajot's data are both "misstated and misconstrued giving an interpretation that the art does not teach."

Finally, the examiner rebuffs Applicant's clinical data on the ground that the statistical comparison between p53-positive and p53-negative cells is invalid in that the groups have different sample sizes. Moreover, even accepting the statistical significance at face value, the examiner argues that the extent of the "surprising result," being limited to that tumor type tested (head & neck), is narrower than the present claims.

A. It is Necessary to Focus on the Claimed Invention in Assessing Obviousness

Applicant has now amended the claims to recite that ***human*** is specifically claimed as previously set forth in dependent claim 6. Claim 1 now reads as follows:

> A method of inhibiting growth of a tumor cell expressing wild-type p53 in a human subject with a solid tumor comprising the steps of:
>
> (a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter; and
>
> (b) paternally administering said viral expression construct to said human subject, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth.

Thus, the following elements of the claim must be suggested by the prior art: first and foremost, one must show treatment of a solid tumor in a human subject; second, the treatment must involve the use of a p53-encoding polynucleotide; third, tumor cells within the tumor must be p53 positive, *i.e.*, express a functional p53 polypeptide; and fourth, the treatment must be demonstrated as inhibiting tumor growth.

Turning to the art, the examiner has cited the following. First is Cajot, which even read as broadly as the examiner argues, shows only *in vitro* studies with p53-positive tumor cells.

Next are Wills and Liu, both of which are cited for *in vivo* animal studies, but only show studies with p53-deficient cells. Finally, Zhang and Bramwell address combination therapies. The propriety of this combination, including assumptions that must be made in order to permit it, are central issues of this appeal. As will be explained, the combination is improper, as is the remaining rejection.

B. There Is No Reasonable Basis for Extrapolating from In Vitro in Animal Studies to Human Clinical Therapy

As argued exhaustively in this case, a key issue is whether the examiner's combination of Cajot with Wills and Liu, as framed above, is proper. More specifically, Applicant submits that the differences between *in vitro* and *in vivo* studies, and the differences between *in vivo* animal models and human clinical applications, especially when simultaneously making the leap from treating p53-negative to p53-positive cells, simply is not warranted.

The Examiner has the burden to show that the *in vitro* and animal studies are reasonably predictive of human clinical studies. The Examiner has not made this case. It is Applicant's position that the cited art that relates to p53-positive cells, which shows only *in vitro* data, cannot be relied upon to predict what would happen in any *in vivo* context, much less in a human gene therapy. The limitations of *in vitro* are manifest, and so well supported in the art that it is unnecessary to recount them here. In fact, the first Office Action addressed limitations on gene therapy, and specifically discussed the shortcomings of delivery and expression of transgenes *in vivo*:

> ... The unpredictability of gene therapy and vector targeting is supported by the teachings of Culver *et al.*, Hodgson *et al.* and Miller *et al.* Culver *et al.*, reviewing gene therapy for cancer, conclude that the "primary factor hampering the widespread application of gene therapy to human disease is the lack of an efficient method for delivering genes *in situ*, and developing strategies to deliver genes to a sufficient number of tumor cells to induce complete tumor regression or restore genetic health remains a challenge" (page 178). Hodgson discusses the drawbacks of viral transduction and chemical transfection

> methods, and states that "[d]eveloping the techniques used in animal models, for therapeutic use in somatic cells, has not been straightforward" (pages 459-460). Miller *et al.* also review the types of vectors available for *in vivo* gene therapy, and conclude that "for all the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances ... targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems" (page 198, column 1).

First Office Action, pages 5-6.

The art is replete with examples of cancer treatments that showed promise *in vitro* only to fail *in vivo*. For example, Planchon *et al.* (1992) showed that butyrate derivatives inhibited growth of breast cancer cell monolayers *in vitro*, but failed to affect the rate of tumor growth *in vivo*. Welters *et al.* (1999), in examining the effects on cisplatin in head & neck cancers, found a lack of correlation between studies on *in vitro* tumor cell lines and *in vivo* tumors. Vingerhoeds *et al.* (1996) similarly compared the effects of doxorubicin on ovarian carcinoma cells and found that *in vitro* inhibition was not observed *in vivo*. Mourad *et al.* (1996) showed that high doses of vitamin A inhibited head & neck and lung cancers *in vitro*, but showed no similar effects *in vivo*. Liu *et al.* (2000) disclosed that, *in vivo*, secretion of TGF-β correlated with resistance to tumor therapy, while no correlation was observed *in vitro*. Finally, Johansson *et al.* (1991) demonstrated that a murine monoclonal antibody inhibited cancer cells *in vitro*, but that *in vivo* inhibition was limited to two days after inoculation into animals, hardly a clinically relevant situation.

Applicant also points out that the Katayose and Srivastava references, as discussed above, not would only fail to support the present rejection if considered, they actually teach ***away***.

Turning to Baker and Casey, the examiner has provided nothing to rebut Applicant's arguments regarding the latter reference. As for Baker, the examiner argues, based on the following equivocal passage, that it actually supports the rejection:

> The transfection and expression results of Table 1 and Fig. 2A suggest that cells at the premalignant stages of tumor progression (VACO 235) may be less sensitive to the inhibitory effects of wild-type p53 than malignant cells (SW480, SW837 and RKO). This hypothesis is consistent with previous results that suggest the wild-type p53 is less inhibitory to the growth of normal rat embryo fibroblasts than to their oncogene-transfected derivatives (8). This sensitivity may only be relative: expression of the wild-type gene at high concentrations might be inhibit the growth of any cell type, including non-neoplastic cells, by overwhelming normal regulatory processes such as phosphorylation.

The examiner's position is fanciful. Somehow, the examiner reads this passage as suggesting gene therapy of p53-positive cells, despite the facts that: (a) the passage notes that premalignant (wtp53) cells are ***not*** as sensitive to p53 transgenes; and (b) normal fibroblast cells (wtp53) are unaffected by p53 trangenes. Worse yet, the last sentence is highlighted by the examiner, but says nothing about a clinical application. Non-specific inhibition of ***any*** cell type, which is all that is proposed in this emphasized statement, would be absolutely ***useless*** since ***all*** cells would be killed, not just tumor cells. By analogy, hydrochloric acid would be a useful chemotherapeutic.

Thus, it is submitted that there is no basis for extrapolating from the *in vitro* studies of Cajot to the *in vivo* studies of Wills and Liu. This is based not only on the well established limitations of *in vitro* systems, but on the clear confusion in the field, as evidenced by Baker, Srivastava, Katayose and Casey.

C. The Cajot Reference is Flawed

Another problem with the rejection is its absolute reliance on Cajot. As has been argued extensively in prior submissions, the Cajot reference cannot be taken on its face due to serious technical flaws in the experimental design. In particular, Applicant has provided evidence that

the SV40 promoter used in the Cajot studies is down-regulated by p53, thereby skewing the results in such a way that the skilled artisan would not rely on the data report therein.

In the Answer, the examiner replied by stating that Subler *et al.* (Exhibit L), which was cited as support for the down-regulation of the SV40 promoter, disclosed that "[t]he SV40 early promoter seems to be the least affected under our assay conditions." While acknowledging that Subler taught inhibition of a variety of cellular and viral promoters, it is argued that those of skill in the art "would be motivated to use the SV40 early promoter to get the highest level of expression possible." Nonetheless, whatever motivation existed, the data flowing from these experiments was flawed and, hence, so would the conclusions drawn therefrom.

Another serious technical flaw exists within Cajot. As discussed in the declaration of Dr. Lou Zumstein, the nude mouse studies described in Cajot. were not true *in vivo* studies. The lung tumor cell lines at issue there were transfected with the p53 vector *ex vivo* (*in vitro*) and only ***then*** injected into the nude mice. Such an assay is not a true *in vivo* assay since one is not establishing tumor *in vivo* first, and then treating the established tumor.

Where one employs an *ex vivo* assay such as was employed by Cajot, there is no test for the effects of the therapy on the tumor *in situ* in the patient's body. Many questions remain unaddressed by such an experiment -- *e.g.*, is the vector capable of penetrating and entering the tumor cells *in situ*; does the therapy have an effect on the tumor mass when the tumor mass is actively growing in an animal (as opposed to mere cells in a test tube); is there sufficient distribution of the vector to cells of the tumor, and sufficient expression within those cells to effect a noticeable growth inhibitory effect; can the material pass through the extracellular matrix that comprises the tumor mass; are there extracellular proteins in the tumor millieu that might block uptake, *etc.*? Studying the effect of a gene such as p53 on cells *in vitro* tells one little

about the ability of a gene to work as a tumor suppressor gene in the clinic, and would not be relevant to the claims at issue in this appeal, which are directed to direct administration to a tumor *in vivo*.

The proper design of a nude mouse tumor assay, where one desires to duplicate an *in vivo* anticancer therapy, is set forth in Ueyama, "Utilization of Nude Mice in Research on Human Cancer," in Animal Models: Assessing the Scope of Their Use in Biomedical Research, 1987 (Exhibit BB). Ueyama extols the importance of using the nude mouse assay in assessing anticancer agents and points out that the assay involves ***first*** establishing the cancer in the nude mouse by xenotransplantation, and ***then*** treating the resultant tumor with the anticancer agent *in situ* (p. 289). As with gene therapy, it would make little sense to treat the cancer cells in a test tube with the anticancer drug and then transplant the cells into the mouse if one desires to test the clinical effect of the drug on the tumor *in situ*.

The one study presented by Cajot in nude mice (albeit using the flawed *ex vivo* rather than *in vivo* therapy as pointed out above) would not support a conclusion that p53 would be an effective therapy against wild-type p53 tumors. In contrast, the Cajot study would argue ***against*** such a conclusion. In the nude mouse study shown in Cajot's Figure 3, the only transfectant purported to be "wild-type" was the cell line designated "X833.W2." However, it is clear from Figure 1C and from the text that "X833.W2" is not a wild type clone at all -- it expressed a mutant p53: "X833.W2 was shown by Western blot analysis to express what appears to be a mildly truncated form of the p53 protein". Page 6958, col. 2.

Furthermore, there are 17 other allegedly wild-type p53 transfectants reported (see Figure 1A). Inexplicably, no nude mouse growth results are shown with any other wild-type p53 transfectant. Yet, the text explicitly discloses that such growth assays ***were*** conducted with at

least 5 other wild-type p53 transfectants but that these transfectants were ***not*** growth inhibited -- that these wild-type transfectants were ***failures***. See p. 6958, col. 1, last full sentence, and middle of col. 2.

Thus, from the studies reported in Cajot *et al.*, it is evident that out of at least 18 different wild-type p53 clones that were analyzed, only one was reported to exhibit some form of growth suppression -- the other 17 were not reported to show any growth suppression. And this one clone that was allegedly growth suppressed, X833.W2, in fact expressed only a mutant p53. The only reasonable scientific conclusion that one can draw from these studies is that the introduction of the p53 gene into the wild-type p53 lung cancer cell line was ***not*** effective at reducing the growth rate of the lung cancer cells in 17 of the 18 purported "wild-type" transfectants, and the one instance where there did appear to be growth suppression, such suppression could not be attributed to wild-type p53 expression.

D. The Provided Clinical Comparison is Relevant

As submitted in the Brief on Appeal, current clinical data supports a conclusion of surprising and unexpected results in the context of the clinical application of the present invention. The clinical data further provides support for the conclusion that when applied in a clinical context, the invention is applicable in the treatment of wild-type p53 expressing tumors virtually to the same degree as in the context of non-wild-type p53 expressing tumors. This is most surely a surprising and unexpected result.

The examiner argues that, since the p53-positive and p53-negative groups compared in this analysis were not of equal size, there can be no statistical relevance. However, Applicant provided these comparisons not for statistical purpose but as a general response rate comparison. Moreover, since both groups were presented at a ratio (responders v. total tested), the sample size

for each would not matter. In sum, these data show a general trend for response rates between p53-positive and p53-negative tumor cells to be about the same. This is surprising.

E. Other In Vivo Studies Indicate That the Claims Are of Appropriate Scope

The examiner has, in previous actions, focused on the fact that Applicant's claims cover "any and all" tumor types, but that only head & neck cancer data are provided. However, other studies evidence the broad applicability of the present invention. For example, one study involving an *in vivo* model of adenoviral p53 therapy of prostate tumors used wild-type p53 prostate cancer cell line LNCap to establish tumors in the prostates of nude mice. Once established, the prostate tumors were treated with intra-prostatic injection of adenoviral p53. Since it is difficult to measure tumor volumes in this model, and since LNCap cells produce PSA, serum PSA levels, an accepted surrogate for prostate tumor volume, were measured. Adenoviral p53 treatment of this p53 wild-type tumor caused significant reduction of PSA levels, evidencing a reduction in the growth of these p53 wild-type tumors. The actual data is shown in Figure 1 attached to the Zumstein Declaration.

In another study, involving an *in vivo* model of adenoviral p53 therapy of lung tumors, the wild-type p53 lung cancer cell line A549 was used to establish subcutaneous tumors in nude mice. Once established, tumors were treated with intra-tumoral injection of adenoviral p53. Adenoviral p53 injection into this p53 wild-type tumor caused a significant delay in the rate of tumor growth. The data is shown Figure 2 attached to the Zumstein Declaration.

In yet another study, involving an *in vivo* model of adenoviral p53 therapy of cervical tumors, the wild-type p53 cervical cancer cell lines SiHa and MS751 were used to establish subcutaneous tumors in nude mice. Once established, tumors were treated with intra-tumoral injection of adenoviral p53. Adenoviral p53 injection into both of these p53 wild-type tumors

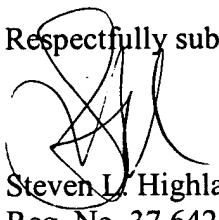
caused a large and significant delay in the rate of tumor growth. The data for SiHa is shown in Figures 3A (single Ad-p53 injection 25 d after tumor cell implantation), 3B (3 injections of Ad-p53 at 25 d post implantation) and 3C (6 injections of Ad-p53 post implantation), attached to the Zumstein Declaration.

Each of these studies shows why the present invention is applicable to a wide variety of cell types and, similarly, why the present claims should not be limited to treatment of head & neck cancers.

V. Conclusion

In light of the preceding, Applicant respectfully submits that all of the remaining claims are non-obvious. Therefore, it is respectfully requested that the Board reverse the remaining grounds for rejection.

Respectfully submitted,



Steven L. Highlander
Reg. No. 37,642

Date: June 5, 2002

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512-418-3000

APPENDIX A: MARKED UP COPY OF AMENDED CLAIMS

1. (Amended) A method of inhibiting growth of a [p53-positive] tumor cell expressing wild-type p53 in a [mammalian] human subject with a solid tumor comprising the steps of:

   (a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter; and

   (b) [directly] parenterally administering said viral expression construct to said [tumor *in vivo*] human subject, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth.

   [wherein said tumor comprises cells that express a functional p53 polypeptide.]

2. (Amended) The method of claim 1 or 146, wherein said tumor is selected from the group consisting of a carcinoma, a glioma, a sarcoma, and a melanoma.

3. (Amended) The method of claim 1 or 146, wherein said tumor cell is malignant.

4. (Amended) The method of claim 1 or 146, wherein said tumor cell is benign.

5. (Amended) The method of claim 1 or 146, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

6. (Amended) The method of claim 1 or 146, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

16. (Amended) The method of claim 1 or 146, wherein the expression construct is injected into a natural or artificial body cavity.

18. (Amended) The method of claim 16, wherein said [contacting is via injection into] body cavity is an artificial body cavity resulting from tumor excision.

19. (Amended) The method of claim 1 or 146, wherein the p53-encoding polynucleotide is tagged so that expression of p53 from said expression vector can be detected.

26. (Amended) The method of claim 1 or 146, wherein said [tumor is contacted with said] expression construct is administered to said tumor at least twice.

28. (Amended) The method of claim 1 or 146, further comprising contacting said tumor with a DNA damaging agent.

36. (Amended) The method of claim 1 or 146, wherein said tumor is located into a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

38. (Amended) The method of claim 11, wherein said [tumor is contacted with said] expression construct is administered to said tumor at least six times within a two week treatment regimen.

146. (New) A method of inducing apoptosis in a tumor cell expressing wild-type p53 in a human subject with a solid tumor comprising the steps of:

(a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide,

wherein said polynucleotide is positioned sense to and under the control of said promoter; and

(b) parenterally administering said viral expression construct to said subject, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth.

147. (New) The method of claim 1 or 146, wherein the expression construct is administered intravenously.

148. (New) The method of claim 1 or 146, wherein the expression construct is administered by direct injection into the tumor.

149. (New) The method of claim 1 or 146, wherein the expression construct is administered intraperitoneally.

150. (New) The method of claim 1 or 146, wherein the expression construct is administered orthotopically.

APPENDIX B: CLEAN COPY OF PENDING CLAIMS (UNOFFICIAL)

1. A method of inhibiting growth of a tumor cell expressing wild-type p53 in a human subject with a solid tumor comprising the steps of:

   (a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter; and

   (b) parenterally administering said viral expression construct to said subject, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth.

2. The method of claim 1 or 146, wherein said tumor is selected from the group consisting of a carcinoma, a glioma, a sarcoma, and a melanoma.

3. The method of claim 1 or 146, wherein said tumor cell is malignant.

4. The method of claim 1 or 146, wherein said tumor cell is benign.

5. The method of claim 1 or 146, wherein said tumor is a tumor of the lung, skin, prostate, liver, testes, bone, brain, colon, pancreas, head and neck, stomach, ovary, breast or bladder.

6. The method of claim 1 or 146, wherein said viral expression construct is selected from the group consisting of a retroviral vector, an adenoviral vector and an adeno-associated viral vector.

7. The method of claim 6, wherein said viral vector is a replication-deficient adenoviral vector.

8. The method of claim 7, wherein said replication-deficient adenoviral vector is lacking at least a portion of the E1-region.

9. The method of claim 8, wherein said promoter is a CMV IE promoter.

11. The method of claim 7, wherein the expression vector is administered to said tumor at least a second time.

12. The method of claim 11, wherein said tumor is resected following at least a second administration, and an additional administration is effected subsequent to said resection.

13. The method of claim 1, wherein said expression vector is administered in a volume of about 3 ml. to about 10 ml.

14. The method of claim 11, wherein the amount of adenovirus in each administration is between about 10^7 and 10^{12} pfu.

16. The method of claim 1 or 146, wherein the expression construct is injected into a natural or artificial body cavity.

17. The method of claim 16, wherein said injection comprises continuous perfusion of said natural or artificial body cavity.

18. The method of claim 16, wherein said body cavity is an artificial body cavity resulting from tumor excision.

19. The method of claim 1 or 146, wherein the p53-encoding polynucleotide is tagged so that expression of p53 from said expression vector can be detected.

20. The method of claim 19, wherein the tag is a continuous epitope.

26. The method of claim 1 or 146, wherein said expression construct is administered to said tumor at least twice.

27. The method of claim 26, wherein said multiple injections comprise about 0.1-0.5 ml volumes spaced about 1 cm apart.

28. The method of claim 1 or 146, further comprising contacting said tumor with a DNA damaging agent.

29. The method of claim 28, wherein said DNA damaging agent is a radiotherapeutic agent.

30. The method of claim 29, wherein said radiotherapeutic agent is selected from the group consisting of γ-irradiation, x-irradiation, uv-irradiation and microwaves.

31. The method of claim 28, wherein said DNA damaging agent is a chemotherapeutic agent.

32. The method of claim 31, wherein said chemotherapeutic agent is selected from the group consisting of adriamycin, 5-fluorouracil, etoposide, camptothecin, actinomycin-D, mitomycin C, verapamil, doxorubicin, podophyllotoxin and cisplatin.

36. The method of claim 1 or 146, wherein said tumor is located into a body cavity selected from the group consisting of the mouth, pharynx, esophagus, larynx, trachea, pleural cavity, peritoneal cavity, bladder interior and colon lumen.

38. The method of claim 11, wherein said expression construct is administered to said tumor at least six times within a two week treatment regimen.

146. A method of inducing apoptosis in a tumor cell expressing wild-type p53 in a human subject with a solid tumor comprising the steps of:

(a) providing a viral expression construct comprising a promoter functional in eukaryotic cells and a polynucleotide encoding a functional p53 polypeptide, wherein said polynucleotide is positioned sense to and under the control of said promoter; and

(b) parenterally administering said viral expression construct to said subject, the administration resulting in expression of said functional p53 polypeptide in cells of said tumor and inhibition of tumor cell growth.

147. The method of claim 1 or 146, wherein the expression construct is administered intravenously.

148. The method of claim 1 or 146, wherein the expression construct is administered by direct injection into the tumor.

149. The method of claim 1 or 146, wherein the expression construct is administered intraperitoneally.

150. The method of claim 1 or 146, wherein the expression construct is administered orthotopically.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: GARY L. CLAYMAN	Group Art Unit: 1632
Serial No.: 08/758,033	Examiner: K. Hauda
Filed: November 27, 1996	Atty. Dkt. No.: INRP:041/HYL
For: METHOD AND COMPOSITION FOR THE DIAGNOSIS AND TREATMENT OF CANCER	

INVENTOR'S DECLARATION UNDER 37 C.F.R. § 1.131

Hon. Commissioner of Patents
Washington DC 20231

I, Gary L. Clayman, declare as follows:

1. I am the sole inventor of the subject matter of all claims currently pending in the referenced patent application.

2. It is my understanding that the Patent and Trademark Office Examiner in charge of the above-captioned application has advanced a rejection of the claims over the references Katayose *et al.*, "Cytotoxic effects of adenovirus-mediated wild-type p53 protein expression in normal and tumor mammary epithelial cells" *Clinical Cancer Research.* 1:889-897 (1995) and Srivastava *et al.*, "Recombinant adenovirus vector expressing wild-type p53 is a potent inhibitor of prostate cancer cell proliferation" *Urology.* 46:843-848 (1995).

3. These articles are in no way relevant to the patentability of my invention, which is directed to the treatment of human cancer patients with the p53 gene. First, both papers cite data from *in vitro* experiments using various cancer cell lines. They do not teach the therapeutic benefits towards humans with the addition of Ad-WTp53. Since the data presented in this invention clearly shows the therapeutic benefits of Ad-WTp53 towards humans, these papers are irrelevant. Secondly, the data is inconsistent further suggesting the unpredictability of human treatment. For example, Srivastava *et al.* showed the addition of Ad-WTp53 negatively affected cell growth *in vitro* but positively affected growth *in vivo*. One is unable to deduce from this contradictory data how Ad-WTp53 would affect human treatment. Furthermore, both papers teach that there are differences between tumor cells that contain mutated p53 and tumor cells that contain wild-type p53. Katayose *et al.* demonstrated that Ad-WTp53 treatment on cells that are deficient of p53 or that contain a mutated p53 gene inhibit cell growth and induce apoptosis. However, they suggest that wild-type p53 tumor cell growth or apoptosis is not affected by the addition of Ad-WTp53. This teaches away from my invention, which is to determine the effects of Ad-WTp53 on wild-type p53 tumor cells.

4. Furthermore, I had fully developed the concept of my invention well before the publication of the forgoing articles. I understand that the earliest of these two articles, Katayose *et al.*, was mailed by the publisher on August 11, 1995. Prior to this date I had fully conceived of using the p53 gene in clinical therapy of human cancer patients having tumors expressing wild-type p53 ("wild-type p53 tumors"). Furthermore, I was diligent in seeking to reduce this invention to practice from August 11, 1995 at least up until the filing of the priority application on November 30, 1995 (US 60/007810).

5. My conception of this invention and diligence is supported by following evidence:

A. Attached as Exhibit 1 is the transcribed tape of my Grand Rounds Seminar dated prior to August 11, 1995 in which I presented my intentions to treat human cancer patients having either wild-type p53 tumors or tumors that contain a mutated p53 gene with an adenovirus carrying wild-type p53 ("Ad-WTp53") (see highlighted text).

B. At that same time, I was working towards obtaining the necessary approvals from the Food and Drug Administration ("FDA"), the National Institutes of Health Recombinant Advisory Committee ("NIH/RAC") and from the institutional review board of MD Anderson Cancer Center ("IRB"), to conduct a clinical trial of the Ad-WTp53 in human cancer patients having both wild-type and mutant p53 tumors.

C. Attached as Exhibit 2 are various approvals received from MD Anderson Cancer Center administration, the IRB and the institutional biosafety committee. While dates have been redacted from these documents, each is dated prior to August 11, 1995.

D. Attached as Exhibit 3 is the final version of the approved informed consent form. This version is redacted to remove names and dates, but this version of the form is dated prior to August 11, 1995.

E. A proposed protocol for the clinical study, designated HNS 94-001, was submitted to the FDA and NIH/RAC prior to August 11, 1995. However, various revisions were required to be made to the protocol during the approval process, and such revisions are entered to the protocol, and dated, on a page-by-page basis. I have attached

the final, approved protocol for HNS 94-001 as Exhibit 4. I should note that all of the dates prior to August 11, 1995 have been redacted from this document. However, an asterisk ("*") has been placed on the upper right corner of each page that is dated prior to August 11, 1995. Pages that were revised after August 11, 1995 are not date-redacted.

F. In this protocol I observe that our laboratory studies had shown that head and neck squamous cell carcinomas ("HNSCC") underwent apoptosis (cell death) when treated with Ad-WTp53, regardless of endogenous p53 status. See Protocol, page 3, second full paragraph. For this reason, the study was designed to include patients having HNSCC regardless of p53 endogenous status of the tumor, and to assess the tumor for its p53 status. See, *e.g.*, Protocol, page 11, section 6.7.

G. On August 24, 1995 I received initial approval from the FDA for the Investigational New Drug ("IND") study with some revisions. See Exhibit 5. The requested revisions were entered into the protocol.

H. On September 25, 1995, I sought administrative approval for the revised protocol. Attached as Exhibit 6 is the approval request with attached revision pages.

I. On October 4, 1995 I received administrative approval for the revised protocol. See Exhibit 7.

J. On October 10, 1995, the revised "Clinical protocol for modification of tumor suppressor gene expression and induction of apoptosis in head and neck squamous cell carcinoma (HNSCC) with an adenovirus vector expressing wildtype p53" (HNS 94-

001) was approved for activation and patient accrual by the Associate Vice President for Clinical and Translational Research (Dr. Leonard A. Zellwing). See Exhibit 8.

K. On October 16, 1995, the surveillance committee report noted that the study had received IRB approval, informed consent approval and FDA approval, but that it was still awaiting NIH/RAC approval. See Exhibit 9.

L. On October 25, 1995, we treated our first patient under the protocol. Treatment of this patient, and enrollment of other patients in the study, continued through the month November of 1995 and beyond, with study close-out occurring in the summer of 1997. See Exhibit 10.

6. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

May 21, 2002
Date

Gary L. Clayman